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Discontinuity between the Voltage-Sensor and the Pore Domain does not Abolish Voltage-Gating of Kv10.1 Potassium Channel
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Voltage-gating of ion channels is crucial for excitable tissues, such as nerve and muscle. Here we show that a voltage-gated potassium channel retains its voltage-dependency of activation, even when the voltage sensor and the pore domain are expressed as two individual proteins from separate cRNAs in Xenopus laevis oocytes. Not only interrupting the S4-S5 cytoplasmic linker at various positions, but also concomitant deletion of several consecutive amino acids from this region yielded functional channels. Moreover, mutations of the voltage-sensor that shift the conductance-voltage curve in either hyperpolarizing or depolarizing direction cause the same shift when the S4-S5 linker is disrupted. Detailed characterization of how the location of discontinuity affects the voltage and time-dependence of activation and deactivation of the split constructs sheds new light on the coupling between the voltage-sensing module and the channel gate.
Our findings indicate that an intact S4-S5 linker is not a sine qua non condition for voltage gating in Kv10.1. In consequence, the idea of direct mechanical coupling between the voltage sensor and the pore mediated by the S4-S5 linker needs to be revised, at least for the KNCH-family channels, which may have a different gating mechanism than Shaker.

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Two KCNQ1 Mutations Associated with Familial Atrial Fibrillation, S140G and V141M, Demonstrate Distinct Voltage Sensor Phenotypes
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KCNQ1 is a voltage-dependent potassium channel that is expressed in the heart with the β-subunit KCNE1 to generate the slowly activating I_{Ks} current that plays a critical role in cardiac impulse conduction by allowing cardiac repolarization. Mutations in KCNQ1 leading to slowing of channel deactivation have been linked to arrhythmias including Short QT syndrome and familial atrial fibrillation. Two adjacent disease-linked mutations located in the voltage sensing domain (S1-S4) of KCNQ1, S140G and V141M, have been shown to drastically slow current deactivation. While their effects on I_{Ks} current deactivation kinetics are similar, their mechanisms may differ. For example, in the absence of KCNE1, S140G but not V141M slows current deactivation. Moreover, crosslinking studies suggest that while V141M can directly interact with KCNE1, S140G cannot. We explore the hypothesis that S140G and V141M, while exhibiting similar effects in I_{Ks} current gating, demonstrate distinct phenotypes on voltage sensor movement. Using voltage clamp fluorometry, we studied voltage sensor movement simultaneously with channel current. We found that in the absence of KCNE1, S140G but not V141M slows voltage sensor deactivation, consistent with their effects on current. Furthermore, in the presence of KCNE1, S140G slows voltage sensor movement, but V141M does not slow voltage sensor deactivation. This work shows that while S140G slows both the current deactivation and voltage sensor movement in the presence and absence of KCNE1, in contrast, V141M slows current deactivation only in the presence of KCNE1, without significantly slowing the kinetics of voltage sensor movement. This suggests that these two mutations slow the deactivation of KCNQ1/KCNE1 channels by different mechanisms, an observation made possible by the simultaneous measurement of both voltage sensor movement and channel current.

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Molecular Determinants of Voltage Sensor Domain Activation
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The voltage sensor domain (VSD) is a four transmembrane segments protein domain that confers voltage sensitivity to ion channels and other proteins. The VSD senses changes in the external potential through its highly positively charged fourth transmembrane segment (S4). The activation mechanism involves a complex, helical screw motion of S4 during which the salt bridge pattern between the S4 arginines and the negative charges of S1-S3 and of the lipid headgroups reorganizes. Together, this ratchet-like motion brings the VSD from the resting to the activated state in a series of jumps that proceed through several intermediate states. We use molecular dynamics simulations and free energy calculations to characterize the free energy landscape and the kinetic rates associated with the different steps of the activation process. To highlight the molecular determinants of this complex conformational transition, we apply techniques of machine learning and data analysis. Specifically, a custom-tailored dimensionality reduction approach is used to extract the relevant degrees of freedom describing the concerted motion of protein residues, lipids and waters.

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Role of the Voltage Sensing Domain S1-S4 in TRPV1 Channels
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Transient Receptor Potential Vallinoid type 1 (TRPV1) channel is a voltage-, heat-, and ligand-activated, nonselective cation channel. As a member of hexahelical cation channel superfamily, TRPV1 does not feature the positively charged S4, which is responsible for the voltage sensitivity of voltage-gated ion channels (VGICs). The origin for TRPV1's voltage sensitivity must lie elsewhere. The objective of this study was to investigate the role of ''classical'' S1-S4 voltage sensor, in particular S4, in the TRPV1, and obtain molecular information about the functional coupling between voltage, chemical and temperature sensors. To test whether the positive charge is involved in activation and to explore the function of the S4 segment in TRPV1 activation, we made a series of single point arginine replacements that scanned residues from E536 through T556 in the S4 and characterized their response to capsaicin, protons and heat using cut-open oocyte voltage-clamp. We also constructed TRPV1/Shaker chimeras in which the S1-S4, S3-S4, or S4 segments of the TRPV1 channel were replaced by the corresponding segments of Shaker channels. The introduction of positively charged residues in the S4 segment resulted in a steeper activation curve and a defective response to capsaicin and protons. Importantly, several of the mutant channels displayed strong inhibition of hyperpolarization-activated inward currents. Three chimeras gave rise to functional channels that exhibited stronger voltage dependence at positive voltages than TRPV1. The chimeras progressively lost inward current with increasing portions replaced by the corresponding Shaker region. They were activated by protons with higher sensitivity compared with TRPV1. These results suggest that re-established outward rectification was dominant over other gating stimuli; in other words, keeping the S1-S4 in the ''deactivated'' state prevented activation by protons, capsaicin or temperature. In wildtype TRPV1, the S4 seems to inhibit activation by protons.

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Sensing the Electrochemical K^+ Gradient: The Voltage Gating Mechanism in K2P Potassium Channels
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Two-pore domain (K2P) K^+ channels represent a large family of ion channels that are major regulators of cellular excitability in the body and involved in a wide range of cellular mechanisms including apoptosis, vasodilatation, anaesthesia, pain, neuroprotection and temperature sensing. Many K2P channels are strongly activated by membrane depolarization, but the mechanisms underlying this voltage-dependent behaviour are unknown. Here we report that many K2P channels (e.g. TREK-1, TREK-2, TRAAK, TASK-3, TALK-2 and TRESK) are equipped with a gating machinery which directly senses the electrochemical K^+ gradient and which gates the pore open when the membrane potential is positive to the K^+ reversal potential. These properties couple voltage activation in K2P channels tightly to the reversal potential in distinction to classical Kv channels. We show that this sensing mechanism is located in the selectivity filter (SF), is strongly affected by the permeant ion species and operates as a check valve that is opened by outward permeation but closed by inward permeation. This gating behaviour is steeply voltage-dependent suggesting that multiple ions within the SF are moved simultaneously by the electrical field to gate the filter open. These findings highlight a mechanism of voltage-dependent gating which bypasses the need for electromechanical coupling to a separate voltage sensing module and is instead powered directly by the electrochemical gradient. This also further closes the mechanistic gap between ion channels and transporters because in both cases the electrochemical gradient is used to power a conformational change leading to either an ion